UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 10 2012

**SUBJECT:** Review of Product Chemistry, Acute Injection Tox/Path study, and waiver request for Toxicity data requirements for section 3 registration of the TGAI: *Bacillus thuringiensis israelensis* strain SUM-6218.

**FROM:** Ibrahim S. Barsoum, Ph.D., Microbiologist Ibrahim S. Barsoum 7-10-2012
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

**THROUGH:** John L. Kough, Ph.D., Senior Scientist John L. Kough
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

**TO:** Denise Greenway, Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

**ACTION REQUESTED:** Review of Product Chemistry, Tox/Path study, and waiver request for Toxicity data requirements submitted by Summit Chemical Co. for registration of the TGAI *Bacillus thuringiensis israelensis* strain SUM-6218.

**CONCLUSION: UPGRADABLE** if the registrant responds to the following deficiencies:

1) The product name and active ingredient content are made to match exactly on the CSF and product label.
2) The deposition of the strain in a recognized microbial culture collection is finalized.
3) The CSF is revised to give a minimum spore or cfu content per unit weight, or a minimum potency in ITU/mg.
4) The information on how the seed culture of *Bacillus thuringiensis israelensis* strain SUM-6218 is maintained prior to its use in the manufacturing process is provided.
5) The CSF is revised to include any preservatives/stabilizers or anti caking agents added to the product, and their MSDSs are provided.
6) Provide an explanation for the presence of the organisms in body organs for a period longer than 21 days or provide an injection tox/path study for a period over 21 days.
7) Submit the results of a one year storage stability study upon completion.

## DATA REVIEW RECORD

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|---|---|
| Active Ingredient: | *Bacillus thuringiensis* subsp. *israelensis* strain SUM-6218 |
| Company Name: | Summit Chemical Co, Baltimore, MD 21224 |
| EPA Reg. No: | 6218-IG |
| Chemical Number: | 6401 |
| Submission Number: | 913558 |
| DP Barcode: | 401187, 401191 |
| MRID No: | 486826-01, 03, 06, 07, & 08: Product Chemistry Studies<br>486826-12: Acute Injection Tox/Path Study<br>486826-13: Hypersensitivity Incidents for TGAI<br>486826-10, 11, 14 – 19: Waiver from Toxicity Data Requirements |

## BACKGROUND:

*Bacillus thuringiensis* subsp. *israelensis* (Bti) was originally isolated by Goldberg and Margalith in Israel in 1977. A study was conducted at the University of Florida to characterize *Bacillus thuringiensis* subsp. *israelensis* strain SUM-6218 with two currently-registered Bti products [ *Bacillus thuringiensis* subsp. *israelensis* strain BMP; and *Bacillus thuringiensis* subsp. *israelensis* strain AM 65-52]. Strain SUM-6218 cells were grown on Lysogeny Broth (LB) plates at 30°C for 48 hours. The cells were stained with crystal violet, followed by Gram iodine and safranine counter stain and observed using light microscopy (100X) for staining and shape. To observe the crystal proteins, cells were fixed on a glass slide by heat and stained with coomassie brilliant blue (0.25% in 50% ethanol and 7% acetic acid) for three minutes, washed with water, and observed using light microscopy. *Bacillus thuringiensis* subsp. *israelensis* strain SUM-6218 has a cream-like color. It is a gram-positive bacillus with a rod shape containing ellipsoid spores that readily stain with coomassie brilliant blue and show motility under a microscope. Cells grown on LB agar in the presence of 100 µg/mL penicillin show that the strain is penicillin resistant.

## DISCUSSION:

The active ingredient in Summit® Bti MP is 100.0% w/w *Bacillus thuringiensis* subsp. *israelensis*, strain SUM-6218, insect delta endotoxin plus byproducts of the fermentation process including *B. thuringiensis israelensis* spores, cell fragments, and spent media from soy flour or other protein sources and minor undigested residues of starches, sugars, fats, proteins, and minerals.x The description of the manufacturing process states that preservatives/stabilizers and an anti caking agent may be added to the product, but these ingredients are not included on the CSF, and MSDSs were not provided. An adequate description of the formation of unintentional ingredients was provided, with the exception that a procedure for disposal of unacceptable batches was not described. Results of a nine-batch analysis are given in terms of ITU/mg, but no potency is given on the CSF or product label. The lower and upper certified limits given for the active ingredient are the OCSPP-recommended limits. The enforcement analytical method is a laboratory

bioassay against *Aedes aegypti* larvae, and provides the results in ITU/mg. The Agency is requesting that the registrant submits a one year storage stability data upon completion and advise the registrant to apply for permit from APHIS to be able to import the bacteria from overseas.

**SUMMARY OF DATA SUBMITTED:**

**Product Chemistry** MRID # 486826-01, 03, 06, 07, & 08

**Product Identity:** The active ingredient in Summit® Bti MP is 100.0% w/w *Bacillus thuringiensis* subsp. *israelensis*, strain SUM-6218, insect delta endotoxin plus byproducts of the fermentation process including spores, cell fragments, and spent media. The CSF gives the active ingredient content as 100.0% w/w; the product label gives it as 100.00% w/w. There are no inert ingredients in the product. *Bacillus thuringiensis* subsp. *israelensis* strain SUM-6218 has a cream-like color. It is a gram-positive bacillus with a rod shape containing ellipsoid spores that readily stain with coomassie brilliant blue and show motility under a microscope. Cells grown on modified egg yolk agar under anaerobic conditions develop white opaque zones of precipitation, indicating that it synthesizes lecithinase.

**Deficiencies:** The product name and active ingredient content must be made to match exactly on the CSF and product label.

**Manufacturing Process:**

[redacted]

**Deficiencies:** Information on how the *Bacillus thuringiensis* subsp. *israelensis* strain SUM-6218 primary culture is maintained prior to its use in the manufacturing process must be provided. If preservatives/stabilizers and/or other inerts are to be added to the product, they must be included on the CSF, with the appropriate adjustments made, and MSDSs or specification sheets for them must be provided.

**Discussion of Formation of Unintentional Ingredients:** [redacted]

[redacted]

**Deficiencies**: Results of testing for ß-exotoxin and mammalian toxicity must be provided to the Agency prior to registration of the product. The procedure to dispose of any unacceptable batches must be described. Also, results of testing for enteric pathogens must be provided.

**Analysis of Samples:** [redacted]

**Deficiencies**: The CSF and product label must be revised to provide a minimum potency of the product in ITU/mg.

**Certification of Limits:** The nominal concentration and certified limits for the ingredients in Summit® Bti MP as given on the CSF. The lower and upper certified limits for the active ingredient are based on the OCSPP-recommended limits (±3%).

**Physical and Chemical Characteristics:**

**Methods**: The methods for determining the applicable physical and chemical characteristics of Summit® Bti MP were not provided. The registrant notes in MRID 48682608 that EPA Forms 8570-36 and 8570-37 should be referred to regarding physical chemical properties.
**Results**: The applicable physical/chemical characteristics of Summit® Bti MP are described.
**Deficiencies:** The registrant should submit the results of a one year storage stability study upon completion.

**Acute Intravenous Injection Toxicity and Infectivity in Rats** MRID # 486826-12

**Methods:** In an acute intravenous injection toxicity and pathogenicity study, groups of young adult Sprague-Dawley rats were injected with Summit Bti MP at a dose of $3.4 \times 10^7$ cfu/animal. The animals were observed for up to 21 days. Five males and five females were treated with autoclaved *Bacillus thuringiensis* subsp./var. *israelensis* as inactive MPCA controls and five males and five females were not treated as untreated controls.
**Results:** All animals survived, gained weight, and appeared normal during the study. No observable abnormalities were noted in any animal at necropsy. Kidney weight of both males and females was significantly decreased relative to untreated controls in Summit Bti MP-treated animals, and brain and liver weight were significantly decreased in treated males. No test organism was detected in the brain tissue of the treated animals in group 3, and the test organism was cleared from the blood and kidneys by day 7. Test organism counts were near zero in the lungs from days 7 through 21. The test organism was not cleared from the liver, spleen, mesenteric lymph nodes, or caecum contents within the 21 days of the study. The caecum count peaked at day 7 ($4.5 \times 10^3$) and

decreased thereafter. The CFU count in the liver increased between days 3 and 7 and remained fairly steady thereafter. Test organism counts in the spleen declined through day 7 but increased thereafter, reaching $6.3x10^2$ cfu/mL by day 21, while test organism counts in the mesenteric lymph nodes declined to 0 cfu/mL by day 14 and then increased to $2.4x10^2$ cfu/mL by day 21. The resurgence of CFUs in the spleen and mesenteric lymph nodes after steadily declining for 7 to 14 days is not a typical pattern. Such results may imply that there exists a reservoir of live bacteria elsewhere in the body. Summit Bti MP does not appear to be toxic or pathogenic in rats when dosed intravenously at $3.4 \times 10^7$ cfu/animal. However, further explanation of the results or an additional study over a period longer than 21 days is needed to determine if Summit Bti MP is infective in rats.
**Deficiencies:** The investigators need to substantiate their statement with literature references or other verifiable information that the bacterial enumeration results in the Group 3 animals reflect an immune recognition and a pattern of clearance that does not imply infectivity in the rat.

**Waiver from Toxicity Data Requirements** MRID # 486826-10, 11, 14 – 19

The registrant gave the following rationale for a waiver request for toxicity studies:

1. Summit's *Bacillus thuringiensis* subsp. *israelensis* (Bti) MP is a manufacturing use product intended for formulation into end-use products (EPs) that are not meant to be applied to agricultural food, feed, or livestock with the possible exception that livestock may drink water treated with Bti end-use products intended for mosquito larvae control and that Bti end-use products may be used to control pest Diptera larvae that infest mushroom culture.

2. Inert ingredients are not likely to pose any significant human health risks because they consist of byproducts of the fermentation process including *Bacillus thuringiensis israelensis* spores, cell fragments, and spent media from soybean flour and minor undigested residues of starches, sugars, fats, proteins, and minerals.

3. These studies are only required for registration of a microbial pesticide when the following conditions apply: The potential to cause adverse human health effects or the product characterization indicates the microbial pesticide has a significant potential to produce a mammalian toxin. Results from toxicity testing have indicated no more adverse effects from *Bacillus thuringiensis* subsp. *israelensis*, than other subspecies, varieties, and/or strains of *Bacillus thuringiensis* registered by the Agency and described in the 1998 EPA Reregistration Eligibility Decision (RED) for *Bacillus thuringiensis*, which includes subsp. *israelensis*.

4. No known mammalian health effects have been demonstrated in any toxicity/pathogenicity studies involving *Bacillus thuringiensis*-Based Pesticides. The sum total of all toxicology data submitted to the Agency complete with the lack of any reports of significant human health hazards of the various *Bacillus thuringiensis* strains allow the conclusion that all toxicity studies be waived as long as product identity and manufacturing process testing data indicated there is no mammalian toxicity associated with the strains.

**CONCLUSION: ACCEPTABLE**